REMARKS

Regarding the Prosecution History:

Applicants are thankful for the Examiner's diligent efforts to advance this application to allowance and are pleased to have this opportunity to address the Examiner's remaining concerns. Upon careful review of the remarks presented in this reply, the Examiner will agree that the claimed invention is patentable and that this application is in good condition for allowance.

In the non-final Office Action of December 21, 2005, the Examiner objected to claim 17, alleging that it depends from a non-elected claim. Please enter and consider the amendments presented in the Appeal Brief filed January 25, 2005. In that Brief, applicants amended claims 12, 17 and 18 to overcome the Examiner's previous objections.

In the non-final Office Action of December 21, 2005, the Examiner rejected:

I. Claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §U.S.C. §112, second paragraph;

II. Claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §112, first paragraph; and

III. Claims 9 – 10, 12 and 17 under 35 U.S.C §102(b) and (e) over Wong et al. (GB 2 294 692).

Regarding the Claim Amendments presented in this reply:

The amendments to the claims add no new matter. The amendment to claim 9 merely adopts the Examiner's suggestion to put the claim in better form. The amendment to claim 10 finds support in claim 9, from which claim 10 depends. An extra space has been deleted in claim 12. This amendment is supported on page 4, line 9 of the specification.

Regarding Rejection I:

The Examiner should withdraw the rejections of claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §U.S.C. §112, second paragraph.

The phrase "derived from" in claim 9 is clear in the context of the specification. The paragraph from line 15 to line 26 on page 3 actually defines the meaning of "derived" in this context as "mutated." The discussion on page 3, line 28 to page 4, line 15 of the specification elaborates on and even exemplifies the concept of "derived monooxygenases." Morover, the discussion on page 4, line 17 to page 5, line 9 extends the definition to "functional equivalents." The specification, therefore, provides a skilled artisan with a clear understanding of the term "derived monooxygenase."

The phrase "having an amino acid sequence according to SEQ ID NO: 2" in claim 9 has been amended, rendering the rejection moot. Applicants note, however, that contrary to the Examiner's suggestion in the second paragraph of page 5 of the Office action mailed December 21, 2005, the polypeptide does not have the amino acid sequence of SEQ ID NO:2, but rather differs from SEQ ID NO:2 by having at least one functional mutation in at least one of the amino acid sequence regions specified in claim 9, i.e., 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88. Thus, claim 9 relates to an amino acid sequence which carries function mutations and is, therefore, derived from SEQ ID NO:2, but is not identical to it.

Regarding the phrase "exogenous or intermediately formed substrate" in claim 10,[1] claim 10 has been amended so as to render this rejection moot. This amendment makes it clear that claim 10 does not refer to intermediately formed substrates in case the corresponding process of claim 9 does not refer to such intermediately formed substrates. Applicants note that the Examiner has no legal basis to demand process steps for forming intermediate substrates in claim 10, because Applicants are entitled to a scope of protection which adequately reflects their contribution to the art. The process for microbiological oxidation based on cytochrome P450 monooxygenase works just as well with exogenously added substrate as with intermediately formed substrate. It would,

[1] The Examiner's reference to claim 12 on page 5 of the Office action seems to be a typographical error, since claim 12 does not include this phrase.

therefore, not be justified to exclude from the scope of protection those process which involve intermediately formed substrates. The term "intermediately formed" substrate itself is clear to a skilled artisan and denotes a compound which is not added as pre-made, exogenous substrate, but – starting from one or more precursor molecules – is formed by a microorganism (whereby the precursor molecule(s) in turn may be intermediately formed or exogenously added compounds). In this context, the Examiner is directed to page 12, lines 37 – 40, which exemplify the difference between intermediately formed and exogenously added substrate.

The phrase "secondary product thereof" in claim 9[2] would be clear to a skilled artisan. An oxidation product according to claim 9, step b) can further be modified within a recombinant microorganism or a reaction medium and therefore give rise to secondary products thereof. This concept, which is already clear to a skilled artisan, is even briefly exemplified on page 2, lines 40 – 42 of the present specification. This portion of the specification mentions the possibility of further converting the immediate reaction product in the context of a non-enzymatic subsequent or side reaction.

The phrase "functional mutation" in claim 9 is defined in on page 3, lines 20 – 21 as "promoting the oxidation of novel organic substrates." In the context of claim 9, the phrase is also clear that the substrates are N-, O-, or S- heterocyclic mono- or polynuclear aromatic compounds. The specification provides ample information how to identify said functional mutations. The paragraph from page 3, line 41 to page 4, line 2 exemplifies that functional mutations can be obtained by amino acid substitutions. The subsequent paragraph on page 4, lines 4 – 15 provides a link between the phrases "functional mutation" and "functional equivalent," and the concept of functional equivalents is extensively described on page 4, line 17 through page 4, line 9. In particular, functional equivalents are again referred to as showing a "modified substrate profile" (page 4, line 31). Thus, the specification provides a skilled artisan with a clear understanding of the concept of functional mutations.

[2] The Examiner's reference to claim 12 on page 6 of the Office action seems to be a typographical error, since claim 12 does not include this phrase.

Regarding Rejection II:

The Examiner should withdraw the rejection of claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §112, first paragraph.

According to the MPEP, "[d]escription of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces" and as such, a single species may be enough to identify the entire genus (see MPEP 2163.II.A.3.a.ii.).

"The 'written description' requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution" (418 F.3d 1358; 2005). The instant Specification, even with only one example, provides a complete written description. One of ordinary skill in the art would not require undue experimentation to create the instant invention because the art at the time of filing would allow said creation.

Moreover, the Examiner is inappropriately requiring conclusive evidence, whereas applicants have provided enough information in the disclosure for one of ordinary skill in the art to practice the invention. According to accepted principles of patent practice applicants are not merely entitled to the literally disclosed invention. If that were the case, then the scope of protection would always be limited to the disclosed examples. However, applicants are rather entitled to the whole range of embodiments, which are made available by their invention without undue experimentation.

Applicants created functionally mutated P450 BM-3 proteins, which – contrary to native P450 BM-3 – have the ability to produce blue indigo-containing pigment (see: experimental result 1, page 18), verified the produced pigment as indigo (see: experimental result 2, page 19) and used one of the mutated P450 MB-3 proteins in a process for producing indigo from indole (experimental result 3, pages 19 – 20). Moreover, Applicants used a mutated P450 BM-3 protein for the oxidation of 8-methylquinoline, a hetero-aromatic compound (Example 7b, page 22). Thus, Applicants have properly shown that the claimed invention can be practiced. It would be within the skill of a person of ordinary skill in the art to apply the invention to the embodiments

within the scope of the present claims, the rejection under 35 U.S.C §112, first paragraph is in error and should be withdrawn.

Regarding Rejection III:

The Examiner should withdraw the rejection of claims 9 – 10, 12 and 17 under 35 U.S.C §102(b) and (e) over Wong et al. (GB 2 294 692). The Examiner stated that

> Wong et al. … discloses a method of oxidizing N-heterocyclic polynuclear aromatic compound [*sic*] with a modified cytochrom P450 monooxygenase having a mutation corresponding to reside 87 of SEQ ID NO:2. (abstract and pages 4 and 14). Since applicants do not place any limitation on the structure of the monooxygenase derived from SEQ ID NO:2, Examiner takes the position that the monooxygenase of Wong et al. is a cytochrome P450 monooxygenase that is 'derived from *Bacillus megaterium*.[3]

However, neither the specifically indicated abstract or the pages 4 or 14 nor any other part of GB 2 294 692 mentions N-heterocyclic polynuclear aromatic compounds as substrates. To the contrary, the second complete paragraph on page 4 of GB 2 294 692 makes clear the range of substrates of GB 2 294 692.

> According to another aspect of the present invention a mutant of the mono-oxygenase cytochrome P-450cam is provided in which the tyrosine residue at position 96 and/or the cysteine residue at position 334 is replaced by another amino acid residue, which mutant has the property of catalysing the oxidation of any one of the following: polycyclic aromatic hydrocarbons, linear or branched alkanes, diphenyl and biphenyl compounds including halogenated variants of such compounds and halogenated hydrocarbons.[4]

GB 2 294 692 only mentions one heterocyclic compound in Scheme 1, but makes clear in

[3] Page 15, lines 12 – 18 of the Office action mailed December 21, 2005.
[4] Page 4, second complete paragraph of GB 2 294 692.

the specification that its use is limited to that of a protection group, not that of a substrate for the oxidation (page 7, last paragraph):

> Examples of monofunctionalised hydrocarbons are cyclohexyl, cyclopentyl and alkyl derivatives (Scheme 1). The oxidation products of *these* compounds are valuable starting materials for organic synthesis, particularly when produced in a homochiral form. A range of aromatic *protecting groups* are envisaged, e.g. benzyl or naphtyl ether and benzoyl or naphthoyl esters and amids (Scheme 1). Of interest are also benzoxazole groups as carboxyl *protecting groups* and N-benyl oxazolidine groups as aldehyde *protecting groups*. Both can be easily cleaved after the enzymatic oxidation and have previously been described in the literature for the microbial oxidations of aldehydes and acids.[5]

In GB 2 294 692, the heterocyclic compounds serve as protection groups, they are added before the oxidation and removed after the oxidation without affecting the oxidized product of interest. In the present invention, however, the heterocyclic compound is an integral part of the oxidized product, in other words: it actually is the product of interest. Any attempt to remove the heterocyclic portion would destroy the product of the inventive oxidation process.

The same considerations apply with regard to US 6,110,074, which is nearly identical to GB 2 294 692.

For at least these reason, it is respectfully submitted that the present rejection is in error and should be withdrawn.

In Conclusion:

The present application is in condition for allowance. Again, applicants are thankful for the Examiner's diligent efforts to advance this application to allowance, and request favorable action in this matter. In order to facilitate the resolution of any issues

[5] Page 7, last paragraph of GB 2 294 692 (emphasis added).

or questions presented by this paper, the Examiner is welcome to contact the undersigned by phone to further the discussion.

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